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(54) **Kits and compositions for the treatment and repair of defects or lesions in cartilage or bone**

Kit und Zusammensetzung zur Behandlung und Wiederherstellung von Knochen- oder Knorpeldefekten oder Verletzungen

Trousses et méthodes pour le traitement et pour la réparation des lésions ou des défauts de cartilage ou d'os

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- **ANNALS OF SURGERY, vol. 211, no. 3, March 1990, pages 288-294, Philadelphia, PA, US; G.A. KSANDER et al.: "Exogenous transforming growth factor-beta 2 enhances connective tissue formation and wound strength in guinea pig dermal wounds healing by secondary intent"**

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Description

[0001] This invention relates to pharmaceutical compositions for the treatment and repair of defects or lesions in bone or in bone and cartilage in full-thickness defects in joints. More specifically, this invention relates to pharmaceutical compositions for treating defects or lesions (used interchangeably herein) in bone or in bone and cartilage in full-thickness defects in joints and to compositions comprising a matrix containing one or more proliferating agents and a transforming factor to promote proliferation and transformation of cartilage repair cells to form new stable cartilage tissue and to compositions comprising a matrix containing an angiogenic factor to stimulate blood vessel formation and an osteogenic factor to stimulate formation of bone. The compositions of this invention are particularly useful in the treatment of full-thickness defects found in severe osteoarthritis, and in other diseases and traumas that produce cartilage or bone injury.

[0002] Joints are one of the common ways bones in the skeleton are connected. The ends of normal articulated bones are covered by articular cartilage tissue, which permits practically frictionless movement of the bones with respect to one another [L. Weiss, ed., Cell and Tissue Biology (Munchen: Urban and Schwarzenburg, 1988) p. 247].

[0003] Articular cartilage is characterized by a particular structural organization. It consists of specialized cells (chondrocytes) embedded in an intercellular material (often referred to in the literature as the "cartilage matrix") which is rich in proteoglycans, collagen fibrils of predominantly type II, other proteins, and water [Buckwalter et al., "Articular Cartilage: Injury and Repair," in Injury and Repair of the Musculoskeletal Soft Tissues (Park Ridge, Ill.: American Academy of Orthopaedic Surgeons Symposium, 1987) p. 465]. Cartilage tissue is neither innervated nor penetrated by the vascular or lymphatic systems. However, in the mature joint of adults, the underlying subchondral bone tissue, which forms a narrow, continuous plate between the bone tissue and the cartilage, is innervated and vascularized. Beneath this bone plate, the bone tissue forms trabeculae, containing the marrow. In immature joints, articular cartilage is underlined by only primary bone trabeculae. A portion of the meniscal tissue in joints also consists of cartilage whose make-up is similar to articular cartilage [Beaupre, A. et al., Clin. Orthop. Rel. Res. , pp. 72-76 (1986)].

[0004] Two types of defects are recognized in articular surfaces, i.e., full-thickness defects and superficial defects. These defects differ not only in the extent of physical damage-to the cartilage, but also in the nature of the repair response each type of lesion can elicit.

[0005] Full-thickness defects of an articular surface include damage to the hyaline cartilage, the calcified cartilage layer and the subchondral bone tissue with its blood vessels and bone marrow. Full-thickness defects can cause severe pain since the bone plate contains sensory nerve endings. Such defects generally arise from severe trauma or during the late stages of degenerative joint disease, such as osteoarthritis. Full-thickness defects may, on occasion, lead to bleeding and the induction of a repair reaction from the subchondral bone [Buckwalter et al., "Articular Cartilage: Composition, Structure, Response to Injury, and Methods of Facilitating Repair," in Articular Cartilage and Knee Joint Function: Basic Science and Arthroscopy (New York: Raven Press, 1990) pp. 19-56]. The repair tissue formed is vascularized fibrous type of cartilage with insufficient biomechanical properties, and does not persist on a long-term basis [Buckwalter et al. (1990), supra].

[0006] Superficial defects in the articular cartilage tissue are restricted to the cartilage tissue itself. Such defects are notorious because they do not heal and show no propensity for repair reactions.

[0007] Superficial defects may appear as fissures, divots, or clefts in the surface of the cartilage, or they may have a "crab-meat" appearance in the affected tissue. They contain no bleeding vessels (blood spots) such as are seen in full-thickness defects.

Superficial defects may have no known cause, but often they are the result of mechanical derangements which lead to a wearing down of the cartilaginous tissue. Mechanical derangements may be caused by trauma to the joint, e.g., a displacement of torn meniscus tissue into the joint, meniscectomy, a laxation of the joint by a torn ligament, malalignment of joints, or bone fracture, or by hereditary diseases. Superficial defects are also characteristic of early stages of degenerative joint diseases, such-as osteoarthritis. Since the cartilage tissue is not innervated [Ham's Histology (9th ed.) (Philadelphia: J. B. Lippincott Co. 1987), pp. 266-272] or vascularized, superficial defects are not painful. However, although painless, superficial defects do not heal and often degenerate into full-thickness defects.

[0008] It is generally believed that because articular cartilage lacks a vasculature, damaged cartilage tissue does not receive sufficient or proper stimuli to elicit a repair response [Webber et al., "Intrinsic Repair Capabilities of Rabbit Meniscal Fibrocartilage: A Cell Culture Model", (30th Ann. Orthop. Res. Soc., Atlanta, Feb. 1984); Webber et al., J. Orthop. Res., 3, pp. 36-42 (1985)]. It is theorized that the chondrocytes in the cartilaginous tissue are normally not exposed to sufficient amounts of repair-stimulating agents such as growth factors and fibrin clots typically present in damaged vascularized tissue.

[0009] One approach that has been used to expose damaged cartilage tissue to repair stimuli involves drilling or scraping through the cartilage into the subchondral bone to cause bleeding [Buckwalter et al. (1990), supra]. Unfortunately, the repair response of the tissue to such surgical trauma is usually comparable to that observed to take place naturally in full-thickness defects that cause bleeding, viz., formation of a fibrous type of

cartilage which exhibits insufficient biomechanical properties and which does not persist on a long-term basis [Buckwalter et al. (1990), supra].

[0010] A variety of growth factors have been isolated and are now available for research and biomedical applications [see e.g., Rizzino, A., Dev. Biol., 130, pp. 411-422 (1988)]. Some of these growth factors, such as transforming growth factor beta (TGF-β), have been reported to promote formation of cartilage-specific molecules, such as type II collagen and cartilage-specific proteoglycans, in embryonic rat mesenchymal cells in vitro [e.g., Seyedin et al., Proc. Natl. Acad. Sci. USA, 82, pp. 2267-71 (1985); Seyedin et al., J. Biol. Chem., 261, pp. 5693-95 (1986); Seyedin et al., J. Biol. Chem., 262, pp. 1946-1949 (1987)].

[0011] Furthermore, a number of protein factors have been identified that apparently stimulate formation of bone. Such osteogenic factors include bone morphogenetic proteins, osteogenin, bone osteogenic protein (BOP), TGF-βs, and recombinant bone inducing proteins.

[0012] Millions of patients have been diagnosed as having osteoarthritis, i.e., as having degenerating defects or lesions in their articular cartilage. Nevertheless, despite claims of various methods to elicit a repair response in damaged cartilage, none of these treatments has received substantial application [Buckwalter et al. (1990), supra; Knutson et al., J. Bone and Joint Surg., 68-B, p. 795 (1986); Knutson et al., J. Bone and Joint Surg., 67-B, p. 47 (1985); Knutson et al., Clin. Orthop., 191, p. 202 (1984); Marquet, Clin. Orthop., 146, p. 102 (1980)]. And such treatments have generally provided only temporary relief. Systemic use of "chondroprotective agents" has also been purported to arrest the progression of osteoarthritis and to induce relief of pain. However, such agents have not been shown to promote repair of lesions or defects in cartilage tissue.

[0013] To date, treatment of patients suffering from osteoarthritis has been directed largely to symptomatic relief through the use of analgesics and anti-inflammatory agents. Without a treatment that will elicit repair of superficial defects in articular cartilage, the cartilage frequently wears down to the subchondral bone plate. At this phase of the disease, i.e., severe osteoarthritis, the unremitting nature of the pain and the significant compromise of function often dictates that the entire joint be excised and replaced with an artificial joint of metal and/or plastic. Some one-half million procedures comprising joint resection and replacement with an artificial joint are currently performed on knees and hips each year. [See, e.g., Graves, E.J., "1988 Summary; National Hospital Discharge Survey", Advanced Data From Vital and Health Statistics, 185, pp. 1-12 (June 19, 1990)].

[0014] One approach to treating cartilage defects is disclosed in co-owned application WO 92/13565. There, the defect is treated with a matrix material and a proliferation agent which stimulates the proliferation of repair cells in the matrix and the area of the defect. However, that application is not directed to the treatment of bone defects, or full thickness defects involving lesions in both bone and cartilage.

[0015] There is, therefore, a need for treatment of cartilage and bone defects as found in the lesions of severe osteoarthritis and for the treatment of other bone defects.

[0016] The present invention solves the problems referred to above by providing effective therapeutic kits and compositions to induce the repair of lesions in cartilage or bone of humans and other animals. Use of the kits and compositions of this invention also promotes the healing of traumatic lesions and forms of osteoarthritis which would otherwise lead to loss of effective joint function leading to probable resection and replacement of the joint.

[0017] In general outline, the kits of this invention for repairing full-thickness defects in joints comprise a matrix that will be incorporated into the animal tissue and is generally biodegradable which may be used for filling the defect in the bone portion of a full-thickness defect up to the level of the bone-cartilage interface. The matrix comprises angiogenic and osteogenic factors for the repair of the bone portion of the defect. The kit also comprises a membrane, which is impermeable to cells and may be used to cover the matrix filling the bone defect. The membrane is sealed to the edges of the defect at the cartilage-bone junction, e.g., by sealing to the cartilage by thermal bonding using a thermal knife or laser. The kit further comprises a matrix which contains a chondrogenic composition, and which will be incorporated into the animal tissue and is generally biodegradable and may be used to fill the remaining cartilage portion of the defect to the top of the cartilage surface. The matrix containing angiogenic and osteogenic factors may also be applied to any bone defect to promote repair.The compositions of this invention for repairing bone defects that do not involve cartilage are such that the bone defect is filled with a composition comprising a matrix containing angiogenic factor(s) and osteogenic factor(s). The osteogenic factor(s) is packaged in an appropriate delivery system.

[0018] The kits and compositions for the treatment of full-thickness defects can be used during arthroscopic, open surgical or percutaneous procedures. Certain kits of this invention may be used such that after identification of the defect, (1) a composition comprising a matrix containing an angiogenic factor and an osteogenic factor packaged in an appropriate delivery system, e.g., liposomes, is used for filling the bone portion of the defect; (2) a membrane, preferably a biodegradable membrane, which prevents cells from migrating from the bone portion of the defect side to the cartilage defect side, is used by placing it over the matrix in the bone defect and sealing the membrane to the edges of the defect at the cartilage-bone junction; and (3) a composition comprising a matrix, preferably biodegradable, and containing a proliferation agent and a transforming

factor which is packaged in an appropriate delivery system is used for filling the cartilage portion of the defect.

[0019] In this last step, the matrix is bonded to the surface of the cartilage portion of the full-thickness defect, for example, by using an adhesion-promoting factor, such as transglutaminase.

[0020] In order that the invention may be more fully understood, the following detailed description is provided. In the description the following terms are used.

[0021] Angiogenic Factor -- as used herein, refers to any peptide, polypeptide, protein or any other compound or composition which induces or stimulates the formation of blood vessels and associated cells (such as endothelial, perivascular, mesenchymal and smooth muscle cells) and blood vessel-associated basement membranes. In vivo and in vitro assays for angiogenic factors are well-known in the art [e.g., Gimbrone, M. A., et al., J. Natl. Cancer Inst., 52, pp. 413-419 (1974); Klagsbrun, M. et al., Cancer Res., 36, pp. 110-113 (1976); Gross et al., Proc. Natl. Acad. Sci. (USA), 80, pp. 2623-2627 (1983); Gospodarowicz et al., Proc. Natl. Acad. Sci. (USA), 73, pp. 4120-4124 (1976); Folkman et al., Proc. Natl. Acad. Sci. (USA), 76, pp. 5217-5221 (1979); Zetter, B. R., Nature (London), 285, pp. 41-43 (1980) ; Azizkhan, R. G. et al., J. Exp. Med., 152, pp. 931-944 (1980)].

[0022] Arthroscopy -- as used herein, refers to the use of an arthroscope to examine or perform surgery on a joint.

[0023] Bone -- as used herein, refers to a calcified connective tissue primarily comprising a network of deposited calcium and phosphate in the form of hydroxyapatite, collagen (predominantly type I collagen) and bone cells, such as osteoblasts and osteoclasts.

[0024] Bone Repair Cell -- as used herein, refers to a cell which, when exposed to appropriate stimuli, will differentiate and be transformed into a bone cell, such as an osteoblast or an osteocyte, which forms bone. Bone repair cells include perivascular cells, mesenchymal cells, fibroblasts, fibroblast-like cells and dedifferentiated chondrocytes.

[0025] Cartilage -- as used herein, refers to a type of connective tissue that contains chondrocytes embedded in an intercellular material (often referred to as the "cartilage matrix") comprising fibrils of collagen (predominantly type II collagen along with other minor types, e.g., types IX and XI), various proteoglycans (e.g., chondroitinsulfate-, keratansulfate-, and dermatansulfate proteoglycans), other proteins, and water. Cartilage as used herein includes articular and meniscal cartilage. Articular cartilage covers the surfaces of the portions of bones in joints and allows movement in joints without direct bone-to-bone contact, and thereby prevents wearing down and damage to apposing bone surfaces. Most normal healthy articular cartilage is also described as "hyaline", i.e., having a characteristic frosted glass appearance. Meniscal cartilage is usually found in joints which are exposed to concussion as well as movement. Such locations of meniscal cartilage include the tempo-ro-mandibular, sterno-clavicular, acromio-clavicular, wrist and knee joints [Gray's Anatomy (New York: Bounty Books, 1977)].

[0026] Cartilage Repair Cell -- as used herein, refers to a cell which, when exposed to appropriate stimuli, will differentiate and be transformed into a chondrocyte. Cartilage repair cells include mesenchymal cells, fibroblasts, fibroblast-like cells, macrophages and dedifferentiated chondrocytes.

[0027] Cell Adhesion Promoting Factor -- as used herein, refers to any compound or composition, including fibronectin and other peptides as small as tetrapeptides which comprise the tripeptide Arg-Gly-Asp, which mediates the adhesion of cells to extracellular material [Ruoslathi et al., Cell, 44, pp. 517-518 (1986)].

[0028] Chemotactic Agent -- as used herein, refers to any compound or composition, including peptides, proteins, glycoproteins and glycosaminoglycan chains, which is capable of attracting cells in standard in vitro chemotactic assays [e.g., Wahl et al., Proc. Natl. Acad. Sci. USA, 84, pp. 5788-92 (1987); Postlewaite et al., J. Exp. Med., 165, pp. 251-56 (1987); Moore et al., Int. J. Tiss. Reac., XI, pp. 301-07 (1989)].

[0029] Chondrocytes -- as used herein, refers to cells which are capable of producing components of cartilage tissue, e.g., type II cartilaginous fibrils and fibers and proteoglycans.

[0030] Fibroblast growth factor (FGF) -- any member of the family of FGF polypeptides [Gimenez-Gallego et al., Biochem. Biophys. Res. Commun., 135, pp. 541-548 (1986); Thomas et al., Trends Biochem. Sci., 11, pp. 81-84 (1986)] or derivatives thereof, obtained from natural, synthetic or recombinant sources, which exhibits the ability to stimulate DNA synthesis and cell division in vitro [for assays see, e.g., Gimenez-Gallego et al., 1986, supra; Canalis et al., J. Clin. Invest., 81, pp. 1572-1577 (1988)] of a variety of cells, including primary fibroblasts, chondrocytes, vascular and corneal endothelial cells, osteoblasts, myoblasts, smooth muscle and glial cells [Thomas et al., 1986, supra]. FGFs may be classified as acidic (aFGF) or basic (bFGF) FGF, depending on their isoelectric points (pI).

[0031] Matrix -- as used herein, refers to a porous composite, solid or semi-solid substance having pores or spaces sufficiently large to allow cells to populate the matrix. The term matrix includes matrix-forming materials, i.e., materials which can form matrices within a defect site in cartilage or bone. Matrix-forming materials may require addition of a polymerizing agent to form a matrix, such as adding thrombin to a solution containing fibrinogen to form a fibrin matrix. Other matrix materials include collagen, combinations of collagen and fibrin, agarose (e.g., Sepharose®), and gelatin. Calcium phosphate may be used alone or in combination with other matrix materials in treating defects in bones.

[0032] Membrane -- as used herein, refers to any material which can be placed between the bone defect por-

tion and the cartilage defect portion of a full thickness defect and which prevents cell migration and blood vessel infiltration from the bone defect portion into the cartilage defect portion of the full thickness defect. The membranes used in the methods and compositions of this invention for the repair of full thickness defects are preferably biodegradable.

[0033] Osteogenic Factor -- as used herein, refers to any peptide, polypeptide, protein or any-other compound or composition which induces or stimulates the formation of bone. The osteogenic factor induces differentiation of bone repair cells into bone cells, such asosteoblasts or osteocytes. This process may be reached via an intermediary state of cartilage tissue. The bone tissue formed from bone cells will contain bone specific substances such as type I collagen fibrils, hydroxyapatite mineral and various glycoproteins and small amounts of bone proteoglycans.

[0034] Proliferation (mitogenic) Agent -- as used herein, refers to any compound or composition, including peptides, proteins, and glycoproteins, which is capable of stimulating proliferation of cells in vitro. In vitro assays to determine the proliferation (mitogenic) activity of peptides, polypeptides and other compounds are well-known in the art [see, e.g., Canalis et al., J. Clin. Invest., pp. 1572-77 (1988); Gimenez-Gallego et al., Biochem. Biophys. Res. Commun., 135, pp. 541-548 (1986); Rizzino, "Soft Agar Growth Assays for Transforming Growth Factors and Mitogenic Peptides", in Methods Enzymol., 146A (New York: Academic Press, 1987), pp. 341-52; Dickson et al., "Assay of Mitogen-Induced Effects on Cellular Incorporation of Precursors for Scavengers, de Novo, and Net DNA Synthesis", in Methods Enzymol., 146A (New York: Academic Press, 1987), pp. 329-40]. One standard method to determine the proliferation (mitogenic) activity of a compound or composition is to assay it in vitro for its ability to induce anchorage-independent growth of nontransformed cells in soft agar [e.g., Rizzino, 1987, supra]. Other mitogenic activity assay systems are also known [e.g., Gimenez-Gallego et al., 1986, supra; Canalis et al., 1988, supra; Dickson et al., 1987, supra]. Mitogenic effects of agents are frequently very concentration-dependent, and their effects can be reversed at lower or higher concentrations than the optimal concentration range for mitogenic effectiveness.

[0035] Transforming Factor -- as used herein, refers to any peptide, polypeptide, protein, or any other compound or composition which induces differentiation of a cartilage repair cell into a chondrocyte. The ability of the compound or composition to induce or stimulate production of cartilage-specific proteoglycans and type II collagen by cells can be determined by in vitro assays known in the art [Seyedin et al., Proc. Natl. Acad. Sci. USA, 82, pp. 2267-71 (1985); Seyedin et al., Path. Immunol. Res., 7, pp. 38-42 (1987)].

[0036] Transforming Growth Factor Beta (TGF-β) -- any member of the family of TGF-β polypeptides [Derynck, R. et al., Nature, 316, pp. 701-705 (1985); Roberts et al., "The transforming growth factor-β's", In Peptide growth factors and their receptors I (Berlin: Springer Verlag, 1990), p.419)] or derivatives thereof, obtained from natural, synthetic or recombinant sources, which exhibits the characteristic TGF-ß ability to stimulate normal rat kidney (NRK) cells to grow and form colonies in a soft agar assay [Roberts et al., "Purification of Type β Transforming Growth Factors From Nonneoplastic Tissues", in Methods for Preparation of Media, Supplements, and Substrata for Serum-Free Animal Cell Culture (New York: Alan R. Liss, Inc., 1984)] and which is capable of inducing transformation of cartilage repair cells into chondrocytes as evidenced by the ability to induce or stimulate production of cartilage-specific proteoglycans and type II collagen by cells in vitro [Seyedin et al., 1985, supra].

[0037] This invention relates to compositions and kits for treating defects or lesions in cartilage or bone. The compositions of this invention comprise matrices having pores sufficiently large to allow cells to populate the matrices.

[0038] For use in the repair of cartilage as in the cartilage layer in a full-thickness defect, the matrix will also contain a proliferation agent to stimulate the proliferation of cartilage repair cells in-the matrix. Preferably, the proliferation agent also serves as a chemotactic agent to attract cartilage repair cells to the matrix. Alternatively, the matrix may contain a chemotactic agent in addition to the proliferation agent. In one preferred embodiment of this invention, the matrix also contains an appropriate concentration of a transforming factor, the transforming factor being contained within or in association with a delivery system which effects release of the transforming factor at the appropriate time to transform the proliferated cartilage repair cells in the matrix into chondrocytes which produce stable cartilage tissue. The matrix may also contain a cell adhesion promoting factor.

[0039] Matrix materials useful in the methods and compositions of this invention for filling or otherwise dressing the cartilage or bone defects include fibrinogen (activated with thrombin to form fibrin in the defect or lesion), collagen, agarose, gelatin and any other biodegradable material which forms a matrix with pores sufficiently large to allow cartilage or bone repair cells to populate and proliferate within the matrix and which can be degraded and replaced with cartilage or bone during the repair process. In some instances, calcium phosphate containing compounds may be used alone or in combination with other biodegradable matrix materials in treating bone defects.

[0040] The matrices useful in the compositions and kits of this invention may be preformed or may be formed in situ, for example, by polymerizing compounds and compositions such as fibrinogen to form a fibrin matrix. Matrices that may be preformed include collagen (e.g., collagen sponges and collagen fleece), chemically modified collagen, gelatin beads or sponges, a gel-forming

substance such as agarose, and any other gel-forming or composite substance that is composed of a matrix material that will fill the defect and allow cartilage or bone repair cells to populate the matrix, or mixtures of the above.

[0041] In one embodiment of this invention, the matrix is formed using a solution of fibrinogen, to which is added thrombin to initiate polymerization shortly before use. A fibrinogen concentration of 0.5-5 mg/ml of an aqueous buffer solution may be used. Preferably, a fibrinogen solution of 1 mg/ml of an aqueous buffer solution is used. Polymerization of this fibrinogen solution in the defect area yields a matrix with a pore size sufficiently large (e.g., approximately 50-200 μm) so that cartilage or bone repair cells are free to populate the matrix and proliferate in order to fill the volume of the defect that the matrix occupies. Preferably, a sufficient amount of thrombin is added to the fibrinogen solution shortly before application in order to allow enough time for the surgeon to deposit the material in the defect area prior to completion of polymerization. Typically, the thrombin concentration should be such that polymerization is achieved within a few to several (2-4) minutes since exposure of cartilage to air for lengthy periods of time has been shown to cause damage [Mitchell et al., J. Bone Joint Surg., 71A, pp. 89-95 (1989)]. Excessive amounts of thrombin should not be used since thrombin has the ability to cleave growth factor molecules and inactivate them. Thrombin solutions of 10-500 units per ml, and preferably 100 units per ml, of an aqueous buffer solution may be prepared for addition to the fibrinogen solution. In a preferred embodiment of this invention, approximately 20 μl of thrombin (100 U/ml) are mixed with each ml of a fibrinogen solution (1 mg/ml) approximately 200 seconds before filling the defect. Polymerization will occur more slowly if a lower concentration of thrombin is added. It will be appreciated that the amount of thrombin solution needed to achieve fibrin polymerization within 2-4 minutes can be given only approximately, since it depends upon the environmental temperature, the temperature of the thrombin solution, the temperature of the fibrinogen solution, etc. The polymerization of the thrombin-activated matrix solution filling the defect is easily monitored by observing the thrombin-induced polymerization of an external sample of the fibrinogen solution. Preferably, in the compositions and methods of this invention, fibrin matrices are formed from autologous fibrinogen molecules, i.e., fibrinogen molecules derived from the blood of the same mammalian species as the species to be treated. Non-immunogenic fibrinogen from other species may also be used.

[0042] Matrices comprising fibrin and collagen may also be used in the compositions and kits of this invention. In a preferred embodiment of this invention, collagenous matrices are used.

[0043] When collagen is used as a matrix material, sufficiently viscous solutions can be made, e.g., using Collagen-Vliess® ("fleece"), Spongostan®, or gelatine-blood-mixtures, and there is no need for a polymerizing agent. Collagen matrices may also be used with a fibrinogen solution activated with a polymerizing agent so that a combined matrix results.

[0044] Polymerizing agents may also be unnecessary when other biodegradable compounds are used to form the matrix. For example, Sepharose® solutions may be chosen that will be liquid matrix solutions at 39-42°C and become solid (i.e., gel-like) at 35-38°C. The Sepharose should also be at concentrations such that the gel filling the defect has a mesh size to allow bone or cartilage repair cells to freely populate the matrix and defect area.

[0045] In the compositions of this invention used in cartilage repair, one or more proliferation (mitogenic) agents may be added to the matrix solution. The proliferation agent or agents should be present in an appropriate concentration range to have a proliferative effect on cartilage repair cells in the matrix filling the defect. Preferably, the same agent should also have a chemotactic effect on the cells (as in the case of TGF-β); however, a factor having exclusively a proliferative effect may be used. Alternatively, to produce chemotactic cell immigration, followed by induction of cell proliferation, two different agents may be used, each one having just one of those specific effects (either chemotactic or proliferative).

[0046] Proliferation (mitogenic) agents useful in the compositions and kits of this invention for stimulating the proliferation of cartilage repair cells include transforming growth factors ("TGFs") such as TGF-αs and TGF-βs; insulin-like growth factor ("IGF I"); acidic or basic fibroblast growth factors ("FGFs"); platelet-derived growth factor ("PDGF"); epidermal growth factor ("EGF"); and hemopoietic growth factors, such as interleukin 3 ("IL-3") [Rizzino, 1987, supra; Canalis et al., supra, 1988; Growth factors in biology and medicine, Ciba Foundation Symposium, 116 (New York: John Wiley & Sons, 1985); Baserga, R., ed., Cell growth and division (Oxford: IRL Press, 1985); Sporn, M.A. and Roberts, A.B., eds., Peptide growth factors and their receptors, Vols. I and II (Berlin: Springer-Verlag, 1990)]. However, these particular examples are not limiting. Any compound or composition which is capable of stimulating the proliferation of cells as demonstrated by an in vitro assay for cell proliferation is useful as a proliferation agent in this invention. Such assays are known in the art [e.g., Canalis et al., 1988, supra; Gimenez-Gallego et al., 1986, supra; Dickson et al., 1987, supra; Rizzino, 1987; supra].

[0047] Chemotactic agents useful in the compositions and kits of this invention for attracting cartilage repair cells to the cartilage defect include, for example, TGF-βs, FGFs (acid or basic), PDGF, tumor necrosis factors (e.g., TNF-α, TNF-β) and proteoglycan degradation products, such as glycosaminoglycan chains [Roberts et al. (1990), supra; Growth factors in biology and medicine, Ciba Foundation Symposium, 116 (New York, John Wiley & Sons, 1985); R. Baserga, ed., Cell growth

and division (Oxford: IRL Press, 1985)]. Assays to determine the chemotactic ability of polypeptides and other compounds are known in the art [e.g., Postlewaite et al., 1987, supra; Wahl et al., 1987, supra; Moore et al., 1989, supra].

[0048] In a preferred embodiment of this invention, the matrix used in cartilage repair contains TGF-β as the proliferation agent and as the chemotactic agent. In particular, TGF-βI or TGF-βII may be used as the proliferation and chemotactic agent. Other TGF-β forms (e.g., TGF-βIII, TGF-βIV, TGF-βV, etc.) or polypeptides having TGF-β activity [see Roberts, 1990, supra] may also be useful for this purpose, as well as other forms of this substance to be detected in the future, and other growth factors. For use as the proliferation agent and chemotactic agent, TGF-β molecules are dissolved or suspended in the matrix at a concentration of preferably 2-50 ng/ml of matrix solution, and most preferably, 2-10 ng/ml of matrix solution. It will be appreciated that the preferred concentration of TGF-β that will stimulate proliferation of cartilage repair cells may vary with the particular animal to be treated.

[0049] A transforming factor or factors may also be present in the matrix solution used in cartilage repair so that after cartilage repair cells have populated the matrix, the transforming factor will be released into the defect site in a concentration sufficient to promote differentiation (i.e., transformation) of the cartilage repair cells into chondrocytes which form new stable cartilage tissue. Proper timing of the release of the transforming factor is particularly important if the transforming factor can inhibit or interfere with the effectiveness of the proliferation agent [see Roberts et al. (1990), supra].

[0050] Transforming factors useful in the compositions and kits of this invention to promote cartilage repair include any peptide, polypeptide, protein or any other compound or composition which induces differentiation of cartilage repair cells into chondrocytes which produce cartilage-specific proteoglycans and type II collagen. The ability of a compound or composition to induce or stimulate production of cartilage-specific proteoglycans and type II collagen in cells can be determined using assays known in the art [e.g., Seyedin et al., 1985, supra; Seyedin et al., 1987, supra]. The transforming factors useful in the compositions and kits of this invention include, for example, TGF-βs, TGF-αs and FGFs (acid or basic). These transforming factors may be used singly or in combination. In addition, TGF-β may be used in combination with EGF.

[0051] The properly timed release of the transforming factor may be achieved by packaging the transforming factor in or with an appropriate delivery system. Delivery systems useful in the compositions and kits of this invention include liposomes, bioerodible polymers, carbohydrate-based corpuscles, water-oil emulsions, fibers such as collagen which are chemically linked to heparin sulfate proteoglycans or other such molecules to which transforming factors bind spontaneously, and osmotic pumps. Delivery systems such as liposomes, bioerodible polymers, fibers with bound transforming factors and carbohydrate-based corpuscles containing the transforming agent may be mixed with the matrix solution used to fill the defect. These systems are known and available in the art [see P. Johnson and J. G. Lloyd-Jones, eds., Drug Delivery Systems (Chichester, England: Ellis Horwood Ltd., 1987)]. Liposomes may be prepared according to the procedure of Kim et al., Biochem. Biophys. Acta, 728, pp. 339-348 (1983). other liposome preparation procedures may also be used. Additional factors for stimulating chondrocytes to synthesize the cartilage tissue components may be included with the transforming factor in the delivery system.

[0052] In a preferred embodiment of this invention, the matrix used in cartilage repair contains TGF-β as the proliferation and chemotactic agent, and contains TGF-β packaged in a delivery system as the transforming factor. In particular, TGF-βI or TGF-βII may be used as the proliferation and chemotactic agent and as the transforming factor. Other TGF-β forms (e.g., TGF-βIII, TGF-βIV, TGF-βV, etc.) or polypeptides having TGF-β activity (see Roberts, 1990, supra) may also be useful for this purpose, as well as other forms of this substance to be detected in the future, and other growth factors.

[0053] In a preferred embodiment for cartilage repair, a TGF-β concentration of preferably 2-50 ng/ml of matrix solution, and most preferably, 2-10 ng/ml of matrix solution, is used as a proliferation agent and as a chemotactic agent. A substantially higher concentration of TGF-β is also present in a subsequently releasable form in the matrix composition as a transforming factor. Preferably, the subsequent concentration of TGF-β is greater than 200 ng/ml of matrix and, most preferably, is greater than 500 ng/ml of matrix. It will be appreciated that the preferred concentration of TGF-β to induce differentiation of cartilage repair cells may vary with the particular animal to be treated.

[0054] It is necessary to stagger the exposure of the cartilage repair cells to the two concentration ranges of TGF-β, since TGF-β at relatively high concentrations (e. g., greater than 200 ng/ml of matrix solution) may not only transform cartilage repair cells into chondrocytes, but also will inhibit chemotactic attraction of cartilage repair cells; whereas at relatively low concentrations (e. g.; 2-10 ng/ml), TGF-β attracts cartilage repair cells and stimulates their proliferation, but will not induce transformation of cartilage repair cells into chondrocytes which produce cartilage tissue.

[0055] In a preferred embodiment of this invention, in order to obtain the sequence of chemotaxis and proliferation, followed by transformation, TGF-β is present both in a free, unencapsulated form and in an encapsulated, or otherwise sequestered, form in the matrix. Preferably, for the purpose of attracting and inducing proliferation of cartilage repair cells in the matrix and defect area, TGF-β molecules are dissolved or suspended in the matrix at a concentration of 2-10 ng/ml of matrix so-

lution. To promote transformation of cartilage repair cells in the matrix into chondrocytes, TGF-β molecules are also present in the matrix sequestered in multivesicular liposomes according to the method of Kim et al., 1983, supra, at a concentration of greater than 200 ng/ml of matrix solution, and preferably at a concentration of greater than 500 ng/ml. The TGF-β-loaded liposomes are disrupted when the attracted cartilage repair cells have populated the matrix and have started to degrade the matrix. During the degradation of the matrix, the cartilage repair cells ingest and/or degrade the liposomes, resulting in the release of TGF-β at concentrations sufficient to induce the transformation of cartilage repair cells into chondrocytes.

[0056] The required two-stage delivery of chemotactic and proliferating versus transforming concentrations of TGF-β may also be achieved by combining transforming concentrations of TGF-β with a bioerodible polymer. Alternatively, a pump, and preferably an implanted osmotic pump, may be used to control the concentration of TGF-β in the defect and matrix. In this embodiment of the invention, the pump controls the concentration of TGF-β in the matrix, i.e., the pump may release TGF-β at an initial chemotactic and proliferation stimulating concentration and at a subsequent transforming concentration. Preferably, the transforming concentration of TGF-β is delivered by the pump approximately 1 to 2 weeks post-operatively. Delivery of the transforming factor into the defect volume is preferably localized to the matrix in the defect site.

[0057] The proliferation agents and, when used, the transforming factors in the compositions of this invention are applied in the defect site within the matrix. Their presence is thus restricted to a very localized site. This is done to avoid their free injection or infusion into a joint space. Such free infusion may produce the adverse effect of stimulating the cells of the synovial membrane to produce joint effusion.

[0058] In the compositions of this invention used in bone repair, one or more angiogenic factors is added to the matrix solution to stimulate the formation and ingrowth of blood vessels and associated cells (e.g., endothelial, perivascular, mesenchymal and smooth muscle cells) and of basement membranes in the area of the bone defect. Angiogenic factors useful in the compositions and kits of this invention for stimulating vascularization throughout the deposited matrix in the area of the bone defect include bFGF, TGF-β, PDGF, TNF-α, angiogenin or angiotropin. Heparin sulfate has been found to enhance the angiogenic activity of bFGF. In a preferred embodiment of this invention, bFGF and heparin sulfate are dissolved, suspended or bound in a matrix at a concentration of approximately 10 ng/ml of matrix solution. The preferred concentrations for other angiogenic factors are: 5 ng/ml of matrix solution for TGF-β, 10 ng/ml of matrix solution for TNF-α, and 10 ng/ml of matrix solution for PDGF. However, bFGF in combination with heparin sulfate is the most preferred angiogenic factor among the above named angiogenic factors.

[0059] An osteogenic factor is also present in the matrix solution used in bone repair so that after blood vessels and associated cells have populated the matrix, the osteogenic factor is released into the bone defect site as the matrix is degraded in a concentration sufficient to promote a process leading to the eventual development of osteoblasts and osteocytes. The osteogenic factor is sequestered or packaged in an appropriate delivery system within the matrix-and is released as the matrix is degraded. The delivery systems used in the cartilage repair compositions are useful in the bone repair compositions of this invention, e.g., liposomes or carbohydrate-based corpuscles (see supra). In one embodiment of this invention, the matrix used in bone repair contains TGF-β packaged in a delivery system as the osteogenic factor, at a concentration of 100 ng/ml of matrix solution. Lower and higher concentrations of TGF-β may be used.

[0060] Osteogenic factors useful in the bone repair compositions of this invention include any peptide, polypeptide, protein or any other compound or composition which induces differentiation of bone repair cells into bone cells, such as osteoblasts and osteocytes, which produce bone tissue. The osteogenic factors useful in this invention include proteins such as TGF-β [Sampath, T. R. et al., J. Biol. Chem., 265(22), pp. 13198-13205 (1990)], osteogenin [Luyten, F. P. et al., J. Biol. Chem., 264(15), pp. 13377-80 (1989)], bone morphogenic protein (BMP) [Wang, E. et al., Proc. Natl. Acad. Sci. USA, 87, pp. 2220-24 (1990)], and TGF-β combined with epidermal growth factor (EGF).

[0061] The differentiation of mesenchymal cells induced by an osteogenic factor may include the formation of intermediary tissues such as fibrous, hyaline and calcified cartilage; and endochondral ossification, which leads to the formation of woven bone tissue, which will become remodelled and transformed into mature lamellar bone tissue. In some instances, bone may be formed directly from mesenchymal cells without the appearance of an intermediary tissue. Within the matrix, the process of bone tissue formation usually occurs 3 to 4 weeks after blood vessels have formed and infiltrated the matrix in response to the angiogenic factor present in the matrix.

[0062] The matrix compositions described in this invention for repairing the bone portion of a full-thickness defect in joints are also useful in treating any defect in bone tissue as is desirable. Such defects include bone fractures, joint fractures, non-unions and delayed unions, percutaneous arthrodesis, pseudo-arthrosis and bone defects resulting from congenital defects, trauma, tumor infection, degenerative disease and other causes of loss of skeletal tissue. The bone repairing matrix compositions are also useful for prosthesis implantation and enhancement of prosthesis stability, enhancement of osseointegration of implant materials used for internal

fixation procedures, stabilization of dental implant materials, healing acceleration of ligament insertion, and spine or other joint fusion procedures.

[0063] Fibronectin or any other compound, including peptides as small as tetrapeptides, that contain the amino acid sequence Arg-Gly-Asp, may be used as cell adhesion promoting factors [Ruoslathi et al., Cell, 44, pp. 517-18 (1986)] in order to enhance the initial adhesion of cartilage or bone repair cells to a matrix deposited in a defect site. Fibrin and certain collagen matrices already contain this sequence [Ruoslathi et al., 1986, supra]. When other biodegradable matrices are used, such cell adhesion promoting factors may be mixed with the matrix material before the matrix is used to fill or dress the defect. Peptides containing Arg-Gly-Asp may also be chemically coupled to the matrix material (e.g., to its fibers or meshes) or to a compound added to the matrix, such as albumin.

[0064] The compositions hereinbefore described are useful in inducing cartilage or bone formation at a selected site of defect in cartilage or bone tissue of an animal.

[0065] The kits and compositions of this invention allow for a treatment of cartilage and bone defects in animals, including humans, that is simple to administer and is restricted in location to an affected joint area. The entire treatment may be carried out by arthroscopic, open surgical or percutaneous procedures.

[0066] In using the kits and compositions for treating defects or lesions in cartilage or bone according to this invention, a defect or lesion is identified, prepared, and filled with the matrix compositions according to this invention.

[0067] In the case of repairing a defect in bone tissue, an angiogenic factor is present in the bone repair composition at an appropriate concentration to stimulate formation of blood vessels within the matrix filling the bone defect. As blood vessels are formed, the osteogenic factor is released from its delivery system to induce the process of bone formation.

[0068] For cartilage repair, a proliferation (mitogenic) agent is present in the matrix composition at an appropriate concentration to stimulate the, proliferation of cartilage repair cells in the matrix and defect or lesion. The same agent may also, at this concentration, serve as a chemotactic agent to attract cartilage repair cells, provided that the factor used has a combined effect with respect to cell proliferation and chemotaxis (as does TGF-β at 2-10 ng/ml of matrix). Alternatively, two different agents may be present in the matrix, one with a specific proliferative effect, and the other with a specific chemotactic effect. In an alternative embodiment, after the defect area is dressed with the matrix, the proliferation agent and, if desired, a chemotactic agent, may be injected directly into the matrix-filled defect area.

[0069] In a subsequent step of cartilage repair, the cartilage repair cells in the matrix are exposed to a transforming factor at the appropriate time at a concentration sufficient to transform the cartilage repair cells into chondrocytes which produce stable cartilage tissue. This may be accomplished by including an appropriate delivery system containing the transforming factor within the matrix composition as described above. Alternatively, the transforming agent may be delivered by injection directly into the matrix-filled defect area at the appropriate time. The transforming concentration should be made available to the cells approximately 1 to 2 weeks following the initial implantation of the matrix into the defect area. Additional factors may be added to the delivery system or directly injected in order to better promote synthesis of the cartilage matrix components at this time point.

[0070] Cartilage or bone defects in animals are readily identifiable visually during arthroscopic examination of the joint or during simple examination of the lesion or defect during open surgery. Cartilage or bone defects may also be identified inferentially by using computer aided tomography (CAT scanning) X-ray examination, magnetic resonance imaging (MRI) analysis of synovial fluid or serum markers, or by any other procedure known in the art.

[0071] The kits and compositions of this invention may be used such that the bone defect site of a full-thickness defect is filled up to the calcified cartilage layer at the bone-cartilage interface with a bone repair matrix composition such that a flat plane is formed. Thereafter, the membrane of the kit, preferably a biodegradable membrane, which is impermeable to cells (e.g., pore sizes less than 5 μm), is placed over the matrix-filled bone defect, and the edges of the membrane sealed to the perimeter of the defect in the region of the cartilage-bone junction. Preferably, the membrane is sealed to the cartilage at the junction by thermal bonding using a thermal knife or laser. The matrix composition comprises a matrix material, an angiogenic factor, and an osteogenic factor, which is packaged in an appropriate delivery system.

[0072] The purpose of the membrane is to prevent blood vessels from infiltrating the layer of cartilage in the case of a full-thickness defect. The formation of blood vessels in the cartilage stimulates bone formation in the cartilage and inhibits complete repair of the cartilage layer. If only a bone defect needs to be repaired, no membrane has to be applied.

[0073] After the membrane has been placed over the matrix-filled bone defect and sealed to the perimeter of the defect in the region of the cartilage-bone junction, the remaining portion of the defect is completely filled with a matrix composition used to stimulate cartilage repair. The composition for cartilage repair comprises a matrix material and a proliferation agent and, if desired, a chemotactic agent. The composition used in this step may also contain, packaged in an appropriate delivery system, a transforming factor. In the most preferred composition or kit for cartilage repair of the invention, the matrix contains a proliferation agent, a chemotactic

agent (which nay be identical to the proliferation agent) and a transforming factor which is packaged in or associated with a delivery system that releases the transforming factor, at a time that the repair cells populating the matrix have begun remodelling the intercellular substance, at a concentration that transforms the cartilage repair cells into chondrocytes. Preferred compositions are described above.

[0074] The adhesion of a matrix to cartilage in a superficial defect or to the cartilage portion of a full-thickness defect can be enhanced by treating the cartilage defect with transglutaminase [see, e.g., Ichinose et al., J. Biol. Chem., 265 (3), pp. 13411-14 (1990); Najjar, V. A. and Lorand, L., eds. Transglutaminases (Boston: Martinus-Nijhoff, 1984). In this embodiment of the invention, the cartilage defect is dried, e.g. by using cottonoid, and filled with a solution of transglutaminase. The solution is then removed, e.g., by suction, leaving a film containing transglutaminase on the cartilage. The defect is then filled with a matrix composition described above for cartilage repair.

[0075] Additional details and examples describing kits and compositions for the treatment and repair of defects in cartilage are described in a commonly owned U.S. patent application, Serial No. 648,274.

[0076] In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes and are not to be construed as limiting this invention in any manner.

EXAMPLE

Repair Of Full-Thickness Defects In Articular Cartilage

[0077] Full-thickness articular cartilage defects, 0.7 mm in width, were created in the medial condyles and patellar grooves of adult mini-pig knee joints. Lesions were effected in a group of four animals maintained under general anaesthesia, using a planing instrument. The vertical extensions of each lesion into the subchondral bone (containing blood vessels and bone marrow cells) was controlled macroscopically by the occurrence of bleeding to insure that a full-thickness lesion had been made in the joint. The depth of the defect was filled in with a collagenous matrix, containing free TGF-β at a concentration of about 4 ng/ml of matrix solution, and liposome-encapsulated TGF-β at a concentration of about 100 ng/ml of matrix volume. This osteogenic matrix composition was applied up to the cartilage-bone junction, at which level a cellulose membrane (pore size 0.2 μm), well adapted to the perimeter of the cartilage-bone junction of the defect area, was inserted. The remaining defect space was filled up to the surface level of the adjacent articular cartilage with a chondrogenic matrix composition as described in this application at page 15, lines 15-21; page 16, lines 7-11; and page 22, lines 1-17.

[0078] About ten weeks after the operation and treatment, the animals were killed and the knee joint components chemically fixed in buffered glutaraldehyde (4%) solutions containing 2.5% Cetyl pyridinium chloride. Following dehydration, in a graded series of increasing ethanol concentration, and embedding in methylmethacrylate, histologic sections were produced and stained with McNeil Tetrachrome and Toluidine Blue O in preparation for light microscopic examination.

[0079] That part of the defect space corresponding in level to the subchondral bone, i.e., where osteogenic matrix had been placed, was fully filled with newly-formed bone tissue. Likewise, the defect space adjacent to articular cartilage tissue, i.e., in the region above the cellulose membrane filled with the chondrogenic matrix composition, was filled with articular cartilage repair tissue.

Claims

1. The use of a composition comprising:

   a matrix or matrix-forming material used to fill a defect in bone;
   an angiogenic factor; and
   an osteogenic factor associated with a delivery system;
   for the preparation of a medicament to treat defects in bone.

2. The use of a composition according to claim 1, wherein the angiogenic factor is selected from the group consisting of bFGF, a mixture of bFGF and heparin sulfate, TGF-β, PDGF, TNF-α, angiogenin, angiotropin and combinations thereof.

3. The use of a composition according to claim 1, wherein the osteogenic factor is selected from the group consisting of TGF-β, a mixture of a TGF-β and EGF, osteogenin, BMP and combinations thereof.

4. The use of a composition according to any one of claims 1 to 3, wherein the matrix used to fill the defect area is selected from the group consisting of fibrin, collagen, gelatin, agarose, calcium phosphate containing compounds and combinations thereof.

5. The use of a composition according to claim 1 or 4, wherein the angiogenic factor is bFGF present at a concentration of 5-10 ng/ml in the matrix and the osteogenic factor is TGF-β associated with an appropriate delivery system which provides a concentration of TGF-β of 100 ng/ml of matrix solution.

6. The use of a composition according to any one of claims 1 to 3, wherein the delivery system is select-

ed from the group consisting of liposomes, bioerodible polymers, collagen fibers chemically linked to heparin sulfate proteoglycans, carbohydrate-based corpuscles, and water-oil emulsions.

7. The use of a composition according to claim 5, wherein the matrix comprises collagen and the delivery system comprises liposomes.

8. The use of the composition according to claim 5 or 7, further comprising an amount of heparin sulfate sufficient to enhance the angiogenic activity of the bFGF.

9. A kit for treating full-thickness defects in joints in animals which comprises:

a first matrix containing an angiogenic factor and containiiig an osteogenic factor associated with a delivery system that releases the osteogenic factor;
a membrane which prevents migration of cells from the bone defect, site to the cartilage defect site, which is used for covering the matrix-filled bone portion of the full-thickness defect and may be sealed to the perimeter of the defect in the region of the cartilage-bone junction; and
a second matrix containing a proliferation agent to stimulate proliferation of repair cells, a chemotactic agent and a transforming factor associated with a delivery system that releases the transforming factor.

10. The kit according to claim 9 further comprising transglutaminase which may be used for covering the surface of the cartilage portion of the full-thickness defect prior to dressing the defect or lesion with the second matrix.

11. The kit according to any one of claims 9 or 10, wherein the osteogenic factor, the proliferation agent, and the transforming factor, are TGF-β.

12. The kit according to any one of claims 9 to 11 in which the delivery system for the delivery of the transforming factor and the osteogenic factor is selected from the group consisting of liposomes, bioerodible polymers, collagen fibers chemically linked to heparin sulfate proteoglycans, carbohydrate-based corpuscles, and water-oil emulsions.

13. The kit according to any one of claims 9 to 12 in which the first matrix is selected from the group consisting of fibrin, collagen, gelatin, agarose, and calcium phosphate containing compounds or combinations thereof.

14. The kit according to any one of claims 9 to 13 in which the second matrix is selected from the group consisting of fibrin, collagen, gelatin, agarose, and combinations thereof.

15. The kit according to any one of claims 9 to 14, wherein the first matrix and the second matrix are fibrin which is formed by addition of thrombin to a solution of fibrinogen immediately before filling the defect or lesion with the fibrinogen solution.

16. The kit according to any one of claims 9 to 15, wherein the angiogenic factor is bFGF present at a concentration of 5-10 ng/ml of the first matrix;
the osteogenic factor is TGF-β encapsulated in liposomes and present at a concentration of 100 ng/ml of the first biodegradable matrix;
the proliferation agent and the chemotactic agent are TGF-β present at a concentration of 2-10 ng/ml of the second matrix; and
the transforming factor is TGF-β encapsulated in liposomes and present at a concentration of greater than 200 ng/ml of the second matrix.

17. The kit according to any one of claims 9 to 16, wherein the first and second matrices further contain a cell adhesion promoting factor comprising the tripeptide Arg-Gly-Asp.

18. The kit according to claim 16, wherein the first matrix comprises collagen.

19. The kit according to claim 16, 17 or 18 further comprising transglutaminase which may be used for covering the surface of the cartilage portion of the full-thickness defect prior to dressing the defect or lesion with the second matrix.

20. The kit according to claim 16 or 18, further comprising an amount of heparin sulfate sufficient to enhance the angiogenic activity of the bFGF.

21. The kit according to claim 11, wherein the angiogenic factor is bFGF.

22. A method of preparing a composition for the treatment of defects in bone comprising:

adding to a matrix or matrix-forming material useful for filling a defect in bone an amount of an angiogenic factor, and an amount of an osteogenic factor selected from the group consisting of TGF-β at 100 ng/ml, BMP, osteogenin, and TGF-β combined with epidermal growth factor, which osteogenic factot is associated with an appropriate delivery system.

23. A method for making a kit for treating full-thickness defects in joints in animals comprising:

combining a first matrix or matrix-forming material useful to fill a defect in a bone with an angiogenic factor and an osteogenic factor associated with an appropriate delivery system;
providing a membrane which can be sealed to the perimeter of the cartilage-bone junction of the full-thickness defect to cover the matrix-filled bone portion of the full-thickness defect and prevent the migration of cells from the bone defect site to the cartilage defect site of the full-thickness defect; and
combining a second matrix or matrix-forming material useful to fill the cartilage portion of the full-thickness defect with a proliferation agent, and a chemotactic agent, and a transforming factor associated with an appropriate delivery system.

24. A composition for the preparation of a medicament to treat defects in bone comprising:

a matrix or matrix-forming material used to fill a defect in bone;
an angiogenic factor at an appropriate concentration to stimulate the formation and ingrowth of blood vessels and associated cells in the matrix and the area of the defect; and
TGF-β associated with an appropriate delivery system which provides a concentration of TGF-β of 100 ng/ml of matrix.

25. A composition for the preparation of a medicament to treat defects in bone comprising:

a matrix or matrix-forming material used to fill a defect in bone;
an angiogenic factor; and
BMP associated with an appropriate delivery system.

26. A composition according to claim 24 or 25, wherein the matrix comprises calcium phosphate.

Patentansprüche

1. Verwendung einer Zusammensetzung, umfassend:

eine Matrix oder ein Matrix-bildendes Material, das verwendet wird, um einen Defekt im Knochen zu füllen,
einen angiogenen Faktor; und
einen osteogenen Faktor, verbunden mit einem Einbringungssystem
für die Herstellung eines Medikaments, um Defekte im Knochen zu behandeln.

2. Verwendung einer Zusammensetzung nach Anspruch 1, wobei der angiogene Faktor ausgewählt ist aus der Gruppe bestehend aus bFGF, einem Gemisch von bFGF und Heparinsulfat, TGF-β, PDGF, TNF-α, Angiogenin, Angiotropin und Kombinationen davon.

3. Verwendung einer Zusammensetzung nach Anspruch 1, wobei der osteogene Faktor ausgewählt ist aus der Gruppe bestehend aus TGF-β, einem Gemisch von TGF-β und EGF, Osteogenin, BMP und Kombinationen davon.

4. Verwendung einer Zusammensetzung nach einem der Ansprüche 1 bis 3, wobei die Matrix, die verwendet wird, um den Bereich des Defekts zu füllen, ausgewählt ist aus der Gruppe bestehend aus Fibrin, Kollagen, Gelatine, Agarose, Calciumphosphat enthaltenden Verbindungen und Kombinationen davon.

5. Verwendung einer Zusammensetzung nach Anspruch 1 oder 4, wobei der angiogene Faktor bFGF ist und in einer Konzentration von 5 bis 10 ng/ml in der Matrix vorliegt, und der osteogene Faktor TGF-β ist und verbunden ist mit einem geeigneten Abgabesystem, das eine Konzentration von TGF-β von 100 ng/ml der Matrixlösung bereitstellt.

6. Verwendung einer Zusammensetzung nach einem der Ansprüche 1 bis 3, wobei das Abgabesystem ausgewählt ist aus der Gruppe bestehend aus Liposomen, biologisch abbaubaren Polymeren, Kollagenfasern, die chemisch mit Heparinsulfat-Proteoglycanen verbunden sind, Teilchen auf Kohlenhydratbasis und Wasser-Öl-Emulsionen.

7. Verwendung einer Zusammensetzung nach Anspruch 5, wobei die Matrix Kollagen umfasst und das Abgabesystem Liposomen umfasst.

8. Verwendung der Zusammensetzung nach Anspruch 5 oder 7, weiterhin umfassend eine Menge an Heparinsulfat, die ausreicht, um die angiogene Aktivität von bFGF zu erhöhen.

9. Kit zur Behandlung von Defekten in voller Ausdehnung (durch alle Schichten) in Gelenken von Tieren umfassend:

eine erste Matrix, enthaltend einen angiogenen Faktor und einen osteogenen Faktor, verbunden mit einem Abgabesystem, das den osteogenen Faktor freisetzt;
eine Membran, die die Wanderung von Zellen vom Ort des Knochendefekts zum Ort des Knorpeldefekts verhindert und zum Bedecken des mit Matrix gefüllten Knochenanteils des Defekts in voller Ausdehnung verwendet wird

und die den Umkreis des Defekts im Bereich der Knorpel-Knochengrenzschicht abdichten kann; und

eine zweite Matrix, enthaltend ein Proliferationsmittel, um die Proliferation von Reparaturzellen zu stimulieren, ein chemotaktisches Mittel und einen transformierenden Faktor, verbunden mit einem Abgabesystem, das den transformierenden Faktor freisetzt.

10. Kit nach Anspruch 9, der weiterhin Transglutaminase umfasst, die zum Bedecken der Oberfläche des Knorpelanteils des Defekts in voller Ausdehnung vor dem Verbinden des Defekts oder der Verletzung mit der zweiten Matrix verwendet werden kann.

11. Kit nach einem der Ansprüche 9 oder 10, wobei der osteogene Faktor, das Proliferationsmittel und der transformierende Faktor TGF-β sind.

12. Kit nach einem der Ansprüche 9 bis 11, wobei das Abgabesystem für das Einbringen des transformierenden Faktors und des osteogenen Faktors ausgewählt ist aus der Gruppe bestehend aus Liposomen, biologisch abbaubaren Polymeren, Kollagenfasern, die chemisch mit Heparinsulfat-Proteoglycanen verbunden sind, Teilchen auf Kohlenhydratbasis und Wasser-Öl-Emulsionen.

13. Kit nach einem der Ansprüche 9 bis 12, wobei die erste Matrix ausgewählt ist aus der Gruppe bestehend aus Fibrin, Kollagen, Gelatine, Agarose und Calciumphosphat enthaltenden Verbindungen oder Kombinationen davon.

14. Kit nach einem der Ansprüche 9 bis 13, wobei die zweite Matrix ausgewählt ist aus der Gruppe bestehend aus Fibrin, Kollagen, Gelatine, Agarose und Kombinationen davon.

15. Kit nach einem der Ansprüche 9 bis 14, wobei die erste Matrix und die zweite Matrix Fibrin sind, das durch Zugabe von Thrombin zu einer Lösung von Fibrinogen unmittelbar vor dem Füllen des Defekts oder der Verletzung mit der Fibrinogenlösung gebildet wird.

16. Kit nach einem der Ansprüche 9 bis 15, wobei

der angiogene Faktor bFGF ist und in einer Konzentration von 5. bis 10 ng/ml der ersten Matrix vorliegt;

der osteogene Faktor TGF-β ist und in Liposomen eingeschlossen ist und in einer Konzentration von 100 ng/ml der ersten biologisch abbaubaren Matrix vorliegt;

das Proliferationsmittel und das chemotaktische Mittel TGF-β sind, die in einer Konzentration von 2 bis 10 ng/ml der zweiten Matrix vorliegen; und

der transformierende Faktor TGF-β ist, der in Liposomen eingeschlossen ist und in einer Konzentration von mehr als 200 ng/ml der zweiten Matrix vorliegt.

17. Kit nach einem der Ansprüche 9 bis 16, wobei die erste und zweite Matrix weiterhin einen Faktor enthalten, der die Zelladhäsion fördert und das Tripeptid Arg-Gly-Asp umfasst.

18. Kit nach Anspruch 16, wobei die erste Matrix Kollagen umfasst.

19. Kit nach Anspruch 16, 17 oder 18, der weiterhin Transglutaminase umfasst, die zum Bedecken der Oberfläche des Knorpelanteils des Defekts in voller Ausdehnung vor dem Verbinden des Defekts oder der Verletzung mit der zweiten Matrix verwendet werden kann.

20. Kit nach Anspruch 16 oder 18, der weiterhin eine Menge an Heparinsulfat umfasst, die ausreicht, um die angiogene Aktivität von bFGF zu erhöhen.

21. Kit nach Anspruch 11, wobei der angiogene Faktor bFGF ist.

22. Verfahren für die Herstellung einer Zusammensetzung zur Behandlung von Defekten in Knochen, umfassend:

Hinzufügen einer Menge eines angiogenen Faktors und einer Menge eines osteogenen Faktors, ausgewählt aus der Gruppe bestehend aus TGF-β in einer Konzentration von 100 ng/ml, BMP, Osteogenin und TGF-β kombiniert mit epidermalem Wachstumsfaktor, wobei der osteogene Faktor mit einem geeigneten Abgabesystem verbunden ist, zu einer Matrix oder zu einem Matrix-bildenden Material, das geeignet ist, einen Defekt im Knochen zu füllen.

23. Verfahren für die Herstellung eines Kits zur Behandlung von Defekten in voller Ausdehnung in Gelenken von Tieren, umfassend:

Zusammenbringen einer ersten Matrix oder eines Matrix-bildenden Materials, geeignet zum Füllen eines Defekts im Knochen, mit einem angiogenen Faktor und einem osteogenen Faktor, der mit einem geeigneten Abgabesystem verbunden ist;

Bereitstellen einer Membran, die den Umkreis der Knorpel-Knochengrenzschicht des Defekts in voller Ausdehnung abdichten kann, um den Matrix gefüllten Knochenanteil des Defekts in voller Ausdehnung zu bedecken und die Wanderung von Zellen vom Ort des Knochende-

fekts zum Ort des Knorpeldefekts des Defekts in voller Ausdehnung zu verhindern; und
Zusammenbringen einer zweiten Matrix oder eines Matrix-bildenden Materials, das geeignet ist, den Knorpelanteil des Defekts voller Dicke zu füllen mit einem Proliferationsmittel und einem chemotaktischen Mittel und einem transformierenden Faktor, der mit einem geeigneten Abgabesystem verbunden ist.

24. Zusammensetzung für die Herstellung eines Medikaments, um Defekte in Knochen zu behandeln, umfassend:

eine Matrix oder ein Matrix-bildendes Material, das zum Füllen eines Defekts im Knochen verwendet wird;
einen angiogenen Faktor in einer Konzentration, die geeignet ist, die Bildung und das Einwachsen von Blutgefäßen und assoziierten Zellen in die Matrix und den Bereich des Defekts zu stimulieren; und
TGF-β, verbunden mit einem geeigneten Abgabesystem, das eine Konzentration von TGF-β von 100 ng/ml Matrix bereitstellt.

25. Zusammensetzung für die Herstellung eines Medikaments, um Defekte in Knochen zu behandeln, umfassend:

eine Matrix oder ein Matrix-bildendes Material, das verwendet wird, um einen Defekt im Knochen zu füllen;
einen angiogenen Faktor; und
BMP, verbunden mit einem geeigneten Abgabesystem.

26. Zusammensetzung nach Anspruch 24 oder 25, wobei die Matrix Calciumphosphat umfasst.

Revendications

1. Utilisation d'une composition comprenant:

une matrice ou un matériau formant une matrice utilisé pour combler un défaut dans un os ;
un facteur angiogénique ; et
un facteur ostéogénique associé à un système de délivrance;
pour la préparation d'un médicament pour traiter des défauts dans l'os.

2. Utilisation d'une composition selon la revendication 1, où le facteur angiogénique est sélectionné dans le groupe consistant en bFGF, un mélange de bFGF et de sulfate d'héparine, TGF-β, PDGF, TNF-α, angiogénine, angiotropine et leurs combinaisons.

3. Utilisation d'une composition selon la revendication 1, où le facteur ostéogénique est sélectionné dans le groupe consistant en TGF-β, un mélange de TGF-β et EGF, ostéogénine, BMP et leurs combinaisons.

4. Utilisation d'une composition suivant l'une quelconque des revendications 1 à 3, où la matrice utilisée pour combler la zone du défaut est sélectionnée dans le groupe consistant en fibrine, collagène, gélatine, agarose et composés contenant du phosphate de calcium et leurs combinaisons.

5. Utilisation d'une composition selon la revendication 1 ou 4, où le facteur angiogénique est bFGF présent à une concentration de 5-10 ng/ml dans la matrice, et le facteur ostéogénique est TGF-β associé à un système approprié de délivrance qui procure une concentration de TGF-β de 100 ng/ml de la solution de la matrice.

6. Utilisation d'une composition selon l'une quelconque des revendications 1 à 3, où le système de délivrance est sélectionné dans le groupe consistant en liposomes, polymères bioérodables, fibres de collagène chimiquement liées à des protéoglycanes de sulfate d'héparine, corpuscules à base de carbohydrates et émulsions eau-huile.

7. Utilisation d'une composition selon la revendication 5, où la matrice comprend du collagène et le système de délivrance comprend des liposomes.

8. Utilisation de la composition selon la revendication 5 ou 7, comprenant de plus une quantité de sulfate d'héparine suffisante pour améliorer l'activité angiogénique de bFGF.

9. Kit pour traiter des défauts de pleine épaisseur dans les articulations des animaux qui comprend:

une première matrice contenant un facteur angiogénique et contenant un facteur ostéogénique associé à un système de délivrance qui libère le facteur ostéogénique ;
une membrane, qui empêche la migration des cellules du site du défaut de l'os au site du défaut du cartilage, que l'on utilise pour couvrir la portion d'os remplie de la matrice du défaut de pleine épaisseur et qui peut être scellé au périmètre du défaut dans la région de la jonction cartilage-os ; et
une seconde matrice contenant un agent de prolifération pour stimuler la prolifération des cellules de réparation, un agent chimiotactique et un facteur de transformation associé à un système de délivrance qui libère le facteur de transformation.

10. Kit selon la revendication 9, comprenant de plus une transglutaminase qui peut être utilisée pour couvrir la surface de la portion de cartilage du défaut de pleine épaisseur avant de dresser le défaut ou lésion avec la seconde matrice.

11. Kit selon l'une quelconque des revendications 9 ou 10, où le facteur ostéogénique, l'agent de prolifération et le facteur de transformation sont TGF-β.

12. Kit selon l'une quelconque des revendications 9 à 11, dans lequel le système de délivrance pour la délivrance du facteur de transformation et du facteur ostéogénique est sélectionné dans le groupe consistant en liposomes, polymères bioérodables, fibres de collagène chimiquement liées à des protéoglycanes de sulfate d'héparine, corpuscules à base de carbohydrates et émulsions eau-huile.

13. Kit selon l'une quelconque des revendications 9 à 12, dans lequel la première matrice est sélectionnée dans le groupe consistant en fibrine, collagène, gélatine, agarose, et composés contenant du phosphate de calcium ou leurs combinaisons.

14. Kit selon l'une quelconque des revendications 9 à 13, dans lequel la seconde matrice est sélectionnée dans le groupe consistant en fibrine, collagène, gélatine, agarose et leurs combinaisons.

15. Kit selon l'une quelconque des revendications 9 à 14, où la première matrice et la seconde matrice sont de la fibrine qui est formée par addition de thrombine à une solution de fibrinogène immédiatement avant de combler le défaut ou la lésion par la solution de fibrinogène.

16. Kit selon l'une quelconque des revendications 9 à 15, où le facteur angiogénique est bFGF présent à une concentration de 5-10 ng/ml de la première matrice ;

le facteur ostéogénique est TGF-β encapsulé dans des liposomes et présent à une concentration de 100 ng/ml de la première matrice biodégradable ;

l'agent de prolifération et l'agent chimiotactique sont TGF-β présents à une concentration de 2-10 ng/ml de la seconde matrice ; et

le facteur de transformation est TGF-β encapsulé dans des liposomes et présent à une concentration de plus de 200 ng/ml de la seconde matrice.

17. Kit selon l'une quelconque des revendications 9 à 16, où les première et seconde matrices contiennent de plus un facteur favorisant l'adhérence des cellules, comprenant le tripeptide Arg-Gly-Asp.

18. Kit selon la revendication 16, où la première matrice comprend du collagène.

19. Kit selon la revendication 16, 17 ou 18, comprenant de plus de la transglutaminase qui peut être utilisée pour couvrir la surface de la portion de cartilage du défaut de pleine épaisseur avant de dresser le défaut ou la lésion avec la seconde matrice.

20. Kit selon la revendication 16 ou 18, comprenant de plus une quantité de sulfate d'héparine suffisante pour améliorer l'activité angiogénique de bFGF.

21. Kit selon la revendication 11, où le facteur angiogénique est bFGF.

22. Méthode de préparation d'une composition pour le traitement de défauts dans un os comprenant:

l'addition, à une matrice ou un matériau formant une matrice utile pour combler un défaut dans un os, d'une quantité d'un facteur angiogénique et d'une quantité d'un facteur ostéogénique sélectionné dans le groupe consistant en TGF-β à 100 ng/ml, BMP, ostéogénine, et TGF-β combiné au facteur de croissance épidermique, lequel facteur ostéogénique est associé à un système approprié de délivrance.

23. Méthode de fabrication d'un kit pour traiter des défauts de pleine épaisseur dans les articulations d'animaux comprenant :

la combinaison d'une première matrice ou d'un matériau formant une matrice utile pour combler un défaut dans un os avec un facteur angiogénique et un facteur ostéogénique associé à un système approprié de délivrance ;
une membrane qui peut être scellée au périmètre de la jonction cartilage-os du défaut de pleine épaisseur pour couvrir la portion d'os comblée de la matrice du défaut de pleine épaisseur et prévenir la migration des cellules du site défaut de l'os au site défaut du cartilage du défaut de pleine épaisseur ; et
la combinaison d'une seconde matrice ou matériau formant une matrice utile pour combler la portion de cartilage du défaut de pleine épaisseur par un agent de prolifération et un agent chimiotactique et un facteur de transformation associé à un système approprié de délivrance.

24. Composition pour la préparation d'un médicament pour traiter des défauts dans un os comprenant:

une matrice ou matériau formant une matrice utilisé pour combler un défaut dans l'os ;
un facteur angiogénique à une concentration

appropriée pour stimuler la formation et la croissance de vaisseaux sanguins et des cellules associées dans la matrice et la zone du défaut ; et

TGF-β associé à un système approprié de délivrance qui procure une concentration de TGF-β de 100 ng/ml de la matrice.

25. Composition pour la préparation d'un médicament pour traiter des défauts dans l'os comprenant:

une matrice ou un matériau formant une matrice utilisé pour combler un défaut dans un os ;
un facteur angiogénique ; et
BMP associé à un système approprié de délivrance.

26. Composition selon la revendication 24 ou 25, où la matrice comprend du phosphate de calcium.